In vitro studies on phytochemicals, antioxidants, and cytotoxicity of some medicinal plant extracts against oral, liver and prostate cancer cell lines

SYNOPSIS OF THE THESIS

Submitted by

Lalit Mohan Sharma

Registration No. AU21ZOPhd0068

Under the supervision of

Dr. (Mrs.) Neetu Sharma

In Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

SCHOOL OF BASIC SCIENCES,

FACULTY OF EDUCATION, BASIC SCIENCES AND HUMANITIES, ABHILASHI UNIVERSITY

CHAIL-CHOWK, MANDI (H.P.) – 175045

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DECLARATION

I hereby declare that the dissertation synopsis entitled **"*In vitro* studies on phytochemicals, antioxidants, and cytotoxicity of some medicinal plant extracts against oral, liver, and prostate cancer cell lines"** to be submitted for the partial fulfillment of the degree of philosophy is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associateship or fellowship of similar other titles. It has not been submitted to any other university or institution for the award of any degree or diploma.

Place: Mandi

Date: 26/06/23

Signature of the Scholar

Lalit Mohan Sharma

Forwarded by: Concern supervisor

Place Mandi

Date 26/06/23

Signature of Supervisor

Dr. (Mrs.) Neetu Sharma

Title

***In vitro* studies on phytochemicals, antioxidants, and cytotoxicity of some medicinal plant extracts against oral, liver and prostate cancer cell lines**

Introduction

Free radicals cause oxidative stress, leading to degenerative disorders like Diabetes mellitus, Parkinson's disease, Alzheimer's disease, and Cancer (Xu *et al.,*2017). Cancer is taking most lives and it is happening due to our daily lifestyle (Mathur *et al.,*2015). In this disease some cells of our body divide in an uncontrolled manner (National Cancer Institute, 2021). It accounted for approximately 10 million deaths in 2020, one in six deaths. Breast, prostate, rectum, lung, colon, and liver cancers are the most common types of cancers. Major causes of cancers are low fruit and vegetable diet, high tobacco and alcohol consumption, high body mass index, and lack of physical exercise. Many cancers are curable if they could be detected well in time.

The projected cancer number in India for 2021 was 26.7 million Disability Adjusted Life Years (DALY) and by 2025 it is expected to increase to 29.8 million. The northern region of India is at its highest.

The major cancer sites are

- Lungs (10.6%),
- Breast (10.5%),
- Oral (5.7%),
- Gastric (5.2%),
- Liver (4.6%),
- and cervix (4.3%) (WHO, NCDIR 2020.

According to ICMR-NCDIR 2022, the total number of cancer cases in 2022 was estimated to be 14,61,427. Every 9th person was to be cancerous. Lungs and breasts were the leading sites in men and women respectively. Lymphoid leukemia in childhood (0-14yrs) 29.2% of boys and 24.2% of girls were affected. All cancer cases may increase to 12.8% in 2025 compared to 2020 (Krishnan *et al.,* 2022).

Prostate cancer is the most frequent and second-highest cause of death for the male population in Western countries (Siegel *et al.,* 2012). In the past 30 years, the data has

increased for prostate cancer cases and mortality (Baade *et al.,* 2013). Some common treatments for this cancer are localized Prostate cancer antigen (PCA), Surgery, radiation therapy, chemotherapy, and hormone ablation. Chemotherapy is the best for metastasized and advanced tumors. But still, its efficacy is limited and it also leads to many side effects for patients. So herbal medication is required for such types of cancers (Wang *et al.,* 2012).

Oral squamous cell carcinoma (OSCC) is a major oral cancer. 3.5 billion people are affected by this cancer. India is the second largest country of such cases. Tobacco, betel-quid, unhygienic oral conditions, and alcohol consumption are the common risk aspects of this cancer (Vivek *et al.,* 2020).

The liver is the body's second largest organ and its chief function is to purify toxic chemicals. Its cancer is the sixth most diagnosed and third leading cancer to death. According to WHO and the Global cancer observatory (GCO) in 2020, estimated 905677 persons were diagnosed worldwide and 830180 people died from this cancer (Li *et al*., 2022).

The cancer burden is growing globally, exerting physical, financial, and emotional stress on families and individuals. Many persons in countries are preparing themselves for such types of diseases and are being overburdened finically due to it. Hence there is more medication requirement at low costs. Chemotherapy is an effective way to deal with cancer. But same time it has many side effects. Many previous publications support the view of using traditional plants and their products for the antioxidation and antiproliferation of cancerous cells. The Himalayan zone provides many plants and their products which are used to generate medicines for cancer. In our traditional system of medication like Ayurveda and Homeopathy, there are several medicinal preparations that are used or suggested for a number of health problems (Yogi *et al.,*2017, Kumar *et al*.,2015). There are many herbal plants from which drugs have been extracted. (Jain *et al*., 2015; Gupta *et al.,* 2014). Yet there are many herbal plants in India and in the Himalayas whose pharmacological properties are yet to be evaluated. According to a survey, more than 80 % population in developing countries and about 60% of the world's people use traditional methods and plants to cure cancer-like diseases (Shrestha *et al.,* 2003).

The major phytochemicals of medicinal plants that play an important part in anti-cancerous activities are polyphenols and flavonoids. They have shown activities in arresting the cell cycle of cancerous cells, inducing apoptosis, suppressing cancer cell proliferation, and

preventing oxidative stress (Tao *et al.,*2017). The Himalayan region is home to numerous important medicinal plants. Native people are taking advantage of these valuable products from time immemorial. There are some plants that should be explored for further evaluation as they are neglected so far. These are *Pyrus pashia, Leucas aspera,* and *Phanera vahlii.* Hence current study includes them for their anti-oxidant and antiproliferative activities.

***Pyrus pashia* Buch. -Ham. ex D. Don** (The wild Himalayan pear)

Family-Rosaceae

Local Name-Kainth

Pyrus pashia Buch-Ham ex D. Don, the wild Himalayan pear belonging to the family Rosaceae is commonly called Kainth in Himachal. It is a small tree growing up to an altitude of 2000 m in the Himalayas on the western side. It is the source of food, fuel, fodder, etc. Its flowers are also edible and have medicinal utility. Fresh flowers are cooked into 'raita' and young leaves are cooked as 'kachru'. Shoot tip, leaves, and flowers are also cooked as a vegetable. Plants possess antiproliferative, hepatotoxic, and anti-inflammatory properties. Besides it is used for diarrhea, hair loss, and also as tea beverages by the Monapo community at Twang. Fruits of *Pyrus pashia (*Figure 1.1) are used for dehydration, headaches, fever, gastrointestinal disorders, hysteria, and in epilepsy. Edible flowers are also used in cardiovascular diseases and cancer by many communities.

Figure 1.1 Fruit *Pyrus pashia*

***Leucas aspera* (Willd.) Linn.**

Family: Lamiaceae

Common name- Thumbi, Dronapushi

Leucas aspera Linn. (Fig 1.2) belongs to the family Lamiaceae and is an annual, erect, branched, and stout plant herb that grows about 15-60 cm. distributed throughout India. Species have many different common names depending upon the region where it is found. It is commonly known as Thumbai or Thumba in Hindi. In Sanskrit, it is known as Dronapushpi which is similar to *Leucas cephalotes* known as Mahadrona. Leaves of L. aspera are opposite, short petiolate, obtuse, and narrow at the base and can reach up to 8cm in length and 1.25 cm in breadth. The thick waxy cuticle is traversed with stomata. Stem is quadrangular and scabrid. The inflorescence is verticillaster, with white flowers, small, and directly attached to the base. They are held together in auxiliary whorls. They are complete, irregular, zygomorphic, bisexual, and hypogynous.

Traditionally plant is used for antifungal, antioxidant, antimicrobial, antinociceptive, prostaglandin inhibitory, and cytotoxic activities. It is used to treat snake bites in the Philippines. Also used as an antipyretic and its juice of flowers is used for intestinal worm infections in children.

Fig 1.2 *Leucas aspera* plant

***Phanera vahlii* (Wight & Arn.) Benth. -Taur**

Family: Fabaceae

Synonyms: *Bauhinia vahlii, Bauhinia racemose.*

Common Name: Bauhinia climber, camel's foot climber, Maloo Creeper,

Local Name: Taur

Phanera vahlii (Wight & Arn.) Benth-Taur plant is an evergreen climbing shrub that grows generally on the tops of trees with the help of tendrils. It is found in soil having good moisture and fertility. Its seeds are edible and leaves are cooked as vegetables. It is used as food, fiber, and medicine by local inhabitants of the western Himalayas. Its leaves are used to, prepare platters or '*plates*' and '*dunus'*. White flowers arranged long in large terminal corymbs are used with salt and white pepper for headaches (*Sood et al.,* 2003). They are also used as an aphrodisiac, tonic, and vermifuge, for stomachic, and for cordage for antihyperglycemic, antioxidant, and anti-inflammatory effects.

Fig 1.3 *Phanera vahlii* Flowers

Review of Literature

Pyrus pashia Buch. -Ham. ex D. Don

Pyrus pashia plant has many medicinal properties and some of which have been discovered so far. Ethanolic extract of flowers possesses a high quantity of phenols and many antioxidants including hydroquinone. The new phenolic compound extracted was glycoside 4-o-Z-coumaroylarbutin (Yin *et al.,*2015). Methanolic extract of leaf, bark, and fruit with a fraction of organic solvents such as chloroform, n-butanol, ethyl acetate, and n-hexane Possess flavonoids, alkaloids, cardiac glycosides, and phenols. The fruit extract with chloroform fraction showed the highest antioxidant activity (Sidiqi *et al.,*2015). Hepatotoxicity was induced by CCl_4. Mice were treated with an aqueous extract of *P. pashia* and it significantly reduced the elevated serum levels of serum transaminases, alkaline phosphatase, and bilirubin and increased the level of total protein as compared to CCl_4 treated group (Athokpam *et al.,*2017).

In a study in Arunachal Pradesh, leaf and fruit extracts were prepared in water, methanol, and petroleum ether (PE). Some phytochemicals obtained from the methanolic extract of fruits are 7-Tetradecanal, Octadecanoic acid, Hexadecenoic acid, d-Mannitol, Hexitol, and beta. -D-Xylofuranoses, Pentadecanoic acid, octadecadienoic acid, d-Mannitol, 1-O-(22-hydroxydocosyl), hydroxy-9-octadecenoic acid, Oleoyl chloride, Squalene, Tetracontane, HexatriacontylPenta fluoro propionate, Stigmast-5-en-3-ol, Alpha-Amyrin, Lup-20(29)-en-3-one, Lupeol, and Stigmast-4-en-3-one.

Phytochemicals obtained from methanolic extract of leaves are 2,4,4-trimethyl-3-nitroso1,3-oxazolidine , D-Aldose , Aniline, 2,5-diethoxy , Pentadecanoic acid , hexadecen-1-ol ,octadecadienoic acid , cis-Vaccenic acid , Octadecanoic acid , 7,8,9-Trimethoxy-4,5-dihydro-1Hbenzo[g]indazole , 12-hydroxy-9-octadecenoic acid , 4,8,12,16-Tetramethylheptadecan-4- olide,1-Heptacosanol ,Stigmast-5-en-3-ol, (3.beta.) , 1h-cyclopenta[a]pentalene , Butanoic acid ,Tetrahydro abietic acid , 26-Hydroxycholesterol.

Phytochemicals obtained from PE extract of leaves are 2-Pentadecanone, 6,10,14-trimethyl Pentadecanoic acid, 2-hexadecane-1-ol, 9,12-octadecadienoic acid, Cis-Vaccenic acid, Octadecanoic acid, Ethyl 9-octadecenoate, 4,8,12,16-Tetramethylheptadecan-4-olide,

Hexatriacontylpentafloropropanoate, Tetracontane, Stigmast-5-en-3-ol, (3. beta.), Dl-. alpha. -tocopherol, Phytol, and acetate (Bharali *et al.,*2019).

Ethanol fruit extract found esterified, free and bound phenol compounds. The main esterified, free, and bound form compounds having phenol were chlorogenic acid, catechin, arbutin, procyanidineB2, Hesperidin, Phlorizin, Epicatechin, etc. (Prakash *et al.,*2019). kainth oil from seeds was extracted and studied. The study concluded that oil possesses polyunsaturated fat oil (PUFA)in which linoleic acid is mainly found. Lipidome was analyzed by High-resolution mass spectroscopy (HRMS) to show the presence of trilienoleates in all triacylglycerols. Kainth seed oil was also characterized by Fourier-transformed infrared (FTIR) and Differential scanning calorimetry (DSC) and concluded many nutraceutical chemicals. Including phytosterols and tocopherols with antioxidant properties (Prakash *et al.,*2021). The ethanolic fruit extract of the plant was studied through the Enzyme-linked immune sorbent assay (ELISA) method for the quantization of protein levels of cytokinin (IL-10, IL-6, and TNF-alpha) in lipopolysaccharide-stimulated RAW macrophages. The Carrageenan-induced mice paw edema model was used to determine inflammatory potential and this study concluded that free and bound phenolic contents of fruit extract are rich in epicatechin, catechin, chlorogenic acid, and arbutin, inhibited Pro-inflammatory cytokinin (interleukin 6 and Tumor necrosis factor-alpha) expression and 5-Lipooxygenase, Cyclooxygenase -2 activities. Oral intake of the extract also showed an anti-inflammatory effect in paw tissues by reducing cellular infiltration and decreasing pro-inflammatory cytokinin (Prakash *et al.,*2022).

***Leucas aspera* (Willd.) Linn.**

Genus Leucas possesses many medicinal properties and is used to cure many diseases such as cough, cold, diarrhea, and inflammatory skin disorder. A number of phytochemicals have been isolated from the Leucas species such as flavonoids, coumarins, lignans, steroids, aliphatic long-chain compounds, terpenes, and fatty acids. The plant possesses anti-inflammatory, antimicrobial, analgesic, antidiarrheal, antioxidant, and insecticidal activities (Hemendra *et al.,*2011). It possesses a significant number of tannins, flavonoids, glycosides, and alkaloids, and more antioxidant activity is found in dried powder ethanol extract and, a moderate amount in n-hexane and ethyl acetate extract. Antioxidant properties were studied using DPPH free radical scavenging, ascorbic acid, and gallic acid as standards (Dass *et al.,*2011). It has been

shown hepatoprotective, Anti-filarial, (Kayesh 2013), analgesic (Karthikeyan *et al.,*2010), anti-ulcer (Hiremath *et al.,*2022), antipyretic (Padama *et al.,*2012), anti-venom (Gopi *et al.,*2014), anti-psoriatic (Rios *et al.,*2019), and immunomodulatory, (Augustine *et al.,*2014). It possesses antitumor activities in ethyl acetate extract (Augustine *et al.,*2014). The Plant possesses essential oil in its aerial parts which were isolated through hydro distillation and studied by gas chromatography coupled with mass spectrometry technique. The oil possesses forty-three compounds including β-caryophyllene, 1-octen-3-ol, α- humulene, *epi*-α-bisabolol, α-pinene, and limonene as main compounds. The oil also possesses sesquiterpene hydrocarbons (Joshi 2015). It has many secondary metabolites such as sterols, Tannins, Glycosides, and Saponins having the properties of anticancer drugs (Rehman *et al.,*2016). Nanoparticles in methanolic extracts have a significant effect on PC3 cancer cell lines confirmed through Scanning electron microscopy (SEM) (Mohan *et al.,*2017). Plant aqueous extract silver nanoparticles possess anti-larvicidal properties against Aedes aegypti, Culex, Anopheles, etc. (Elumalai *et al.,*2017).

Leucas aspera roots and leaves extracts were evaluated in aqueous, methanol, and hexane extracts through FTIR analysis, and found various polyphenolic compounds. Plants also possess antioxidant activities studied by DPPH and hydrogen peroxide-free radical scavenging techniques. The plant also possesses anti-microbial activities against various bacteria and fungi. (Vasudha *et al.,*2018). It has anti-diabetic, anti-cancerous, and toxicity-associated activities, studied in Wistar Albino rats and found that due to diabetes medication, lipid peroxidation happens and it causes liver and kidney damage. But hot water extract of the plant has a very potent effect with negligible side effects. The nanoparticles of iron oxide in the plant have shown increased behavior in cancer cells and healthy cells of 'He La' with no toxicity of various doses (Madhu *et al.,*2019).

Ethanolic extract of plant flowers possess anti-tumor activities experimented on mouse peritoneum in vivo model (Jayanthi *et al.,*2020). It has flavonoids that have more anti-oxidants as compared to alkaloids when in vitro cytotoxic viability assay was completed on leaf extracts on MCF 7 cancer cell lines (Patil *et al.,*2021). Leaf extract of the plant was used in green synthesis nanoparticles of copper oxide along with Morinda tinctoria being applied in nano bio-sciences (Radhakrishnan *et al.,*2021). Leaf aqueous extract of the plant was used to, prepare SO_2 nanoparticles confirmed through FTIR, X-ray diffraction analysis (XRD), and

SEM techniques. Nanoparticles were used in the study of 'He La' cervical cancer cell lines in MG63, antibacterial, and in photocatalytic effects on various dyes (Murugnasen *et al.,*2022).

Phanera vahlii (Wight & Arn.) Benth.

Phanera vahlii (Wight &Arn.) Benth, -Taur plant is an evergreen climbing shrub found in soil having good moisture and fertility. It is used as food, fiber, and medicine by local inhabitants of the western Himalayas. Its leaves are used to, prepare platters or '*plates*' and '*dunus'*. Root extracts of *Bauhinia vahlii* were prepared to evaluate antimicrobial activities against gram-positive, and gram-negative bacteria and fungi strains (Dugasani *et al.,*2010). Bark possesses anti-inflammatory and anti-diabetic activities in the stem. The extract showed the presence of flavonoids, phytosterols, alkaloids, glycosides, and phenolic compounds. The ethanolic and chloroform extracts showed a significant reduction in blood glucose levels in rats (Das *et al.,*2012). Methanol extract of plant possesses the highest level of phenols, tannins, and flavonoids. (Sondharajan *et al.,*2013). Plant is traditionally used as medicine for their antioxidant, anti-inflammatory, and antidiabetic activities. The leaves and shoot parts of some species were analyzed including *Phanera vahlii* and found polyphenols, flavonoids, and fatty acids (Frag *et al.,*2015). The Root Extract has carbohydrates, tannins, phenols, saponins, amino acids, proteins, fats, and phytosterols. Also concluded that the roots of this plant possess an ayurvedic drug named Moorva when the properties were compared with the plant Marsdenia tenacissima (Saravanan *et al.,*2015). It was recently segregated into a separate genus, Phenera. Leaves and stems of plants of genus Bauhinia have been used as folk remedies for the treatment of numerous health problems mainly diabetes (Elbanna *et al.,*2016). *Phanera vahlii* fruit biomass (PVF) was studied along with its biochar to eliminate Cr (VI) from synthetic solutions. Techniques used were Brunauer-Emmett-Teller (BET), FTIR, Energy dispersive X-ray microanalysis (EDX), Field emission scanning electron microscopy (FESEM), and Thermogravimetric analysis (TGA) (Ajmani *et al.,*2019). Its different parts like stem, leaves, bark, and seeds are used in different ways by Trible people. Plant checks soil erosion and promotes soil fertility (Kumar *et al.,*2020). It maintained the ecological balance used in the food, medicine, and handicraft industry (Devi *et al.,*2022).

Research Gap identification

After reviewing the literature on *Pyrus pashia* Buch. -Ham. ex D. Don, it is clear that bark, flower, fruit, and leaves have been evaluated in methanol, ethanol, water, and Petroleum ether extracts. All extracts of the plant possess polyphenols, flavonoids, glycosides, etc. It possesses many properties such as anti-inflammatory, antioxidant, hepatoprotective, and antiproliferative against gastric, lung and He La cancer cell lines. Though the above activities have been evaluated, still its antioxidant and antiproliferative activities are yet to be explored in other extracts such as acetone and ethanol extracts, and with these extracts' liver, prostate, and oral cancer lines can be explored.

It is clear from the literature of *Leucas aspera* (Willd.) Linn. that plants possess numerous medicinal properties. Some of them are antioxidant, ant-cancerous, analgesic, anti-asthmatic, and also used for bronchitis, paralysis, jaundice, against helminths worms, etc. Its methanol and ethanol extracts have been verified for various diseases. It is also tested against colorectal cancer cell lines and liver cancer cell lines. But its oxidative and anti-proliferative activities are still to be evaluated against liver, prostate cancer, and oral cancer cell lines. Its acetone extract may be used to evaluate further phytochemicals.

Regarding *Phanera vahlii (Wight & Arn.) Benth.*, its leaves, bark, flower, and roots possess anti-inflammatory, anti-diabetic, and anti-oxidative properties. A literature study tells us that phenols, flavonoids, tannins, and saponins are present in various extracts like methanol, hexane, ethyl alcohol, and acetone. Its acetone, and alcohol extracts along with its anti-cancerous aspect may be explored more as this plant is being used in the handicraft industry for the preparation of platters and people are using this for many years. So, its anti-cancerous aspect must be explored. As the probability of finding more phenols and flavonoids cannot be denied, a study on liver, prostate, and oral cancer cell lines could be explored.

Aims and objectives of the study:

Aims:

To evaluate the phytochemical, antioxidant, and anti-cancerous activities of *Pyrus pashia* (PP), *Leucas aspera* (LA), and *Phanera vahlii* (PV): ***in vitro*** approach.

Objectives:

- Determination of Phytochemical constituents by total phenolic contents.

- Determination of Antioxidant activity by DPPH, ABTS, and hydrogen peroxide scavenging activity.
- Determination of Phytochemical constituents of plant extracts by HPLC techniques.
- Assessment of the cytotoxicity of *Pyrus pashia*, *Leucas aspera*, and *Phanera vahlii* (*In vitro)*.
- Determination of the anti-cancerous activity of plant extracts against cancerous cell lines using MTT assay [3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide].
- Molecular docking study.

Plant Material

Pyrus pashia (fruits), *Leucas aspera* whole plant (wp), and *Phanera vahlii* (flower) will be collected from Hamirpur, Himachal Pradesh, India, and their identification will be confirmed by the Department of Botany, SPU, Mandi.

Plant extract

The aqueous, ethanolic, and acetone extracts of *Pyrus pashia* (fruits), *Leucas aspera (*whole plants), and *Phanera vahlii* (flowers) will be subjected to experimental analysis.

Cell lines:

The following human cancer cell lines will be procured from National Centre for Cell Science (NCCS) Pune.

HepG2 (Liver cancer)	Einbonda *et al.,* 2009
DU145(Prostate cancer)	Yamashita *et al.,*2009
KB-3-1 (Oral cancer)	Walbi *et al.,* 2023

Hypothesis

This investigation aims to prove the antioxidant and anti-cancerous effects of *Pyrus pashia* (PP), *Leucas aspera* (LA), and *Phanera vahlii* (PV) *by* testing these plants against some selective indices of oxidative stress. Therefore, this study will provide a scientific rationale for the traditional use of PP, LC, and PV for the management of cancerous diseases. Thus, the proposed work will help us to evaluate and confirm the efficacy of *Pyrus pashia* (PP), *Leucas aspera* (LA), and *Phanera vahlii* (PV) for the development of more appropriate

anti-cancerous drugs and this will lead to an advantage for the betterment of humankind.

Proposed Research Methodology:

Determination of Phytochemical constituents and antioxidant activity in aqueous, ethanolic, and acetone extracts of *Pyrus pashia* (PP), *Leucas aspera* (LA), and *Phanera vahlii* (PV)

Assessment of Phytochemical constituents: Phytochemical constituents will be determined by total phenolic content and analytical chromatographic technique.

Assessment of antioxidant properties: Antioxidant properties will be determined by DPPH scavenging activity (Shimada *et al*., 1992), ABTS scavenging activity (Re *et al.,*1999) and Hydrogen peroxide scavenging activity (Ruch *et al.,* 1989)

Determination of Phytochemical constituents:

Phytochemical constituents present in plant extracts will be determined by LCMS/HPLC techniques.

Assessment of cytotoxic activity: Assessment of cytotoxic activity of *Pyrus pashia* (fruits), *Leucas aspera* whole plant (wp), and *Phanera vahlii* (flower) by *in vitro* as follows:

- Determination of Cytotoxicity activity of *Pyrus pashia* fruit aqueous extracts at three doses (50, 75,100µg/mL).
- Determination of Cytotoxicity activity of *Pyrus pashia* fruit ethanol extracts at three doses (50, 75,100µg/mL).
- Determination of Cytotoxicity activity of *Pyrus pashia* fruit acetone extracts at three doses (50, 75,100µg/mL).
- Determination of Cytotoxicity activity of *Leucas aspera* wp aqueous extracts at three doses (50, 75,100µg/mL).
- Determination of Cytotoxicity activity of *Leucas aspera* wp ethanol extracts at three doses (50, 75,100µg/mL).
- Determination of Cytotoxicity activity of *Leucas aspera* wp acetone extracts at three doses (50, 75,100µg/mL).

- Determination of Cytotoxicity activity of Phanera *vahlii* flower aqueous extracts at three doses (50, 75,100µg/mL).
- Determination of Cytotoxicity activity of *Phanera vahlii* flower ethanol extracts at three doses (50, 75,100µg/mL).

- Determination of Cytotoxicity activity of *Phanera vahlii* flower acetone extracts at three doses (50, 75,100µg/mL).

Determination of the anti-cancerous activity of plant extracts:

Determination of the anti-cancerous activity of plant extracts by MTT assay [3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide] assay.

Molecular docking study:

Docking experiments will be carried out between the phytochemicals of the effective *Pyrus pashia* , *Leucas aspera* , and *Phanera vahlii* extract and the target protein.

Data Submission and Statistical Analysis

Values will be expressed in mean ± SD for each group. *P* value will be calculated using one-way ANOVA followed by Dunnett's test for multiple comparisons. Values of $p < 0.05$ will be considered significant in all cases.

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